Remarks

Applicants wish to thank the Examiner for the attention accorded to the instant application, and respectfully requests reconsideration of the application as amended. Claims 1-18 are pending in the present application. Claims 1, 4, 5, 8 and 9 have been amended. Support for these amendments can be found throughout the present application generally.

Claim 18 has been added. Claim 18 is patentable for at least the reason that the combination of references cited herein do not teach a process for C-terminal stepwise degradation, which is suitably used for preparation of such a mixture comprising the denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence. A complete analysis of the references is set forth below in greater detail. Support for Claim 18 can be found throughout the application generally, page 75 line 13 to page 82 line 18 specifically. No new matter has been added.

Claim 1 stands rejected under 35 U.S.C. §112, second paragraph, for allegedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1, 2, and 4-6 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Tsugita, Akira et. al., "Additional Possible tools for identification of proteins on one or two dimensional electrophoresis, 1998, Electrophoresis, Vol. 19, pages 928-938 (hereinafter "Tsugita") in view of Covey et. al. U.S Patent No. 5,952,653 (hereinafter "Covey") and Xu, Naxing et. al., "Structural characterization of peptidoglycan muropeptides by matrix-assisted laser desorption ionization mass spectrometry and postsource decay analysis," 1997, Analytical Biochemistry, Vol. 248, page 7-14 (hereinafter "Xu").

Claim 3 stands rejected under 35 U.S.C. 103(a) as being unpatentable over Tsugita in view of Covey and Xu, as applied to Claims 1-2 and 4-6 above, and further in view of Harris, William A., et. al., "Use of matrix clusters and trypsin autolysis fragments as mass calibrants in matrix assisted laser desorption/ionization time-of-flight mass spectrometry," 2002, Rapid Communications in Mass Spectrometry, vol. 16, pages 1714-1722 (hereinafter "Harris").

Claims 7-17 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Tsugita in view of Covey and Xu as applied to 1, 2 and 4-6 above, and further in view of Vogt, S. et. al., "Effective esterification of carboxymethyl cellulose in a new non-aqueous swelling system," 1996, Polymer Bulletin, Vol. 36, page 549-555 (hereinafter "Vogt").

In view of the following remarks, Applicants request further examination and reconsideration of the present patent application.

Rejection under 35 U.S.C. §112

Claim 1 stands rejected under 35 U.S.C. §112, second paragraph, for allegedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 1 has been amended to more clearly recite what Applicant regards as the invention. Specifically Claim 1 has been amended to recite that the highest cationic species peak is the peak identified in Step 2, with the highest anionic species peak being the peak identified in Step 2.

Thus, withdrawal of the rejection of Claim 1 is respectfully requested.

Rejections under 35 U.S.C. §103

Claims 1, 2, and 4-6 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Tsugita in view of Covey and Xu. This rejection should be withdrawn based on the comments and remarks herein.

The combination of Tsugita, Covey and Xu do not render the claimed invention obvious for at least the following reasons.

Tsugita teaches a first step of extracting the protein from the protein spot on the polyacrylamide gel is carried out by using the following extraction procedure.

The protein spot was excised from the polyacrylamide gel and broken up by the use of a small hand-held homogenizer after addition to the 500 μL of 6M guanidine-HCI, 0.1% SDS, 0.5 M Bicine, 4mM EDTA, pH 8.0-8.5. The 6M guanidine-HCI and 0.1% SDS contained in the solution is successfully used to denature the protein, and thus the denatured protein can be easily extracted from the gel carrier to be collect in the pool of supernatant and two addition washes.

The denatured protein contained in the pooled supernatant is subjected to purification and separation with use of a mini-column of C18 silica. The denatured protein isolated by the column separation method is dried up to use as the dried protein sample.

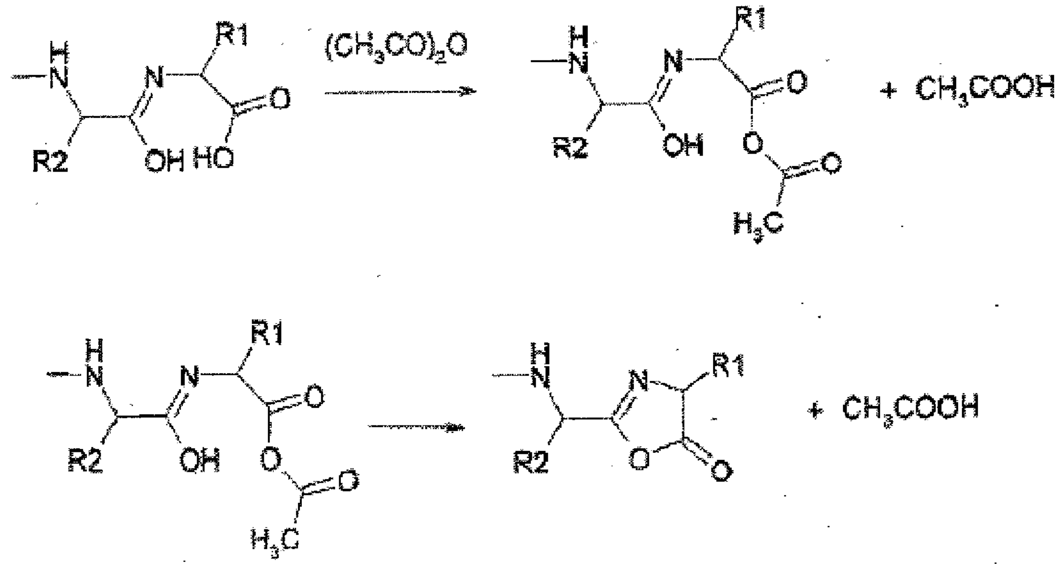
Tsugita fails to teach any process for C-terminal sequencing, in which the reactions for C-terminal stepwise degradation are carried out for the peptide being maintained in a state that it is bound on the gel carrier.

Tsugita teaches such a procedure of reactions for C-terminal stepwise degradation used for the dried protein sample comprising the following three reaction sub-steps (i) - (iii):

(i) The first reaction sub-step for acetylation of the N-terminus of the peptide (denatured protein) and formation of an oxazolone at the C-terminal carboxyl group of the peptide (denatured protein):

Acetic anhydride with 20% acetic acid tetrahydrofuran solution in the present of 1% DTT was reacted on the dried sample of peptide (denatured protein) at 60 °C for 10 min. The reaction of formation of the oxazolone may be carried out by the following reaction scheme:

Formation of the oxazolone at the C-terminal carboxvl group:



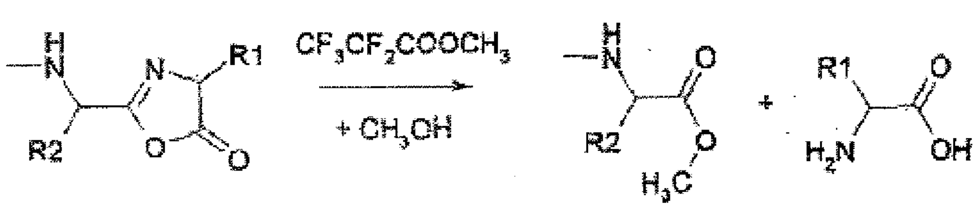
The acetic acid may be used as a catalyst for inducing the convention of keto-form of the amido moiety into the enol-form.

(ii) The second reaction sub-step for degradation of the oxazolone-ring to liberate the C-terminal amino acid and to form the esterified peptide:

The reaction is made with 5% PFPMe (pentafluoropropionic methyl ester: CF_3CF_2-CD-OCH_3) in methanol (CH_30H) at 5 °C for 15 min.

The reaction of degradation of the oxazolone may be carried out by the following reaction scheme:

Degradation of the oxazolone:

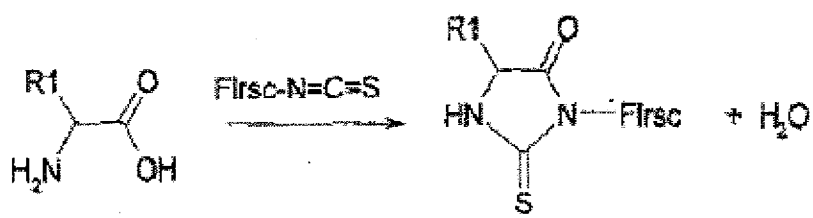


The reaction mechanism may be alcoholysis in help of catalytic function of PFPMe (pentafluoropropionic methyl ester: CF_3CF_2-GO-OCH_3). The C-terminal amino acid was liberated to be dissolved in the methanol solution, and thus, the peptidyl reaction product was formed in the shape of esterified peptide.

Therefore, PFPMe (pentafluoropropionic methyl ester: CF_3CF_2-CO-OCH_3) was used as a catalytic agent for inducing the solvolysis reaction with use of methanol (CH_30H) on the oxazolone-ring.

The C-terminal amino acid isolated in the form of free amino acid was modified with fiuorescien isothiocyanate, and then analyzed by HPLC. The reactron scheme for the modification with fluorescein isothiocyanate may be shown as follows.

<u>Modification with fluorescien isothiocvanate (FIrsc-N=C=S):</u>



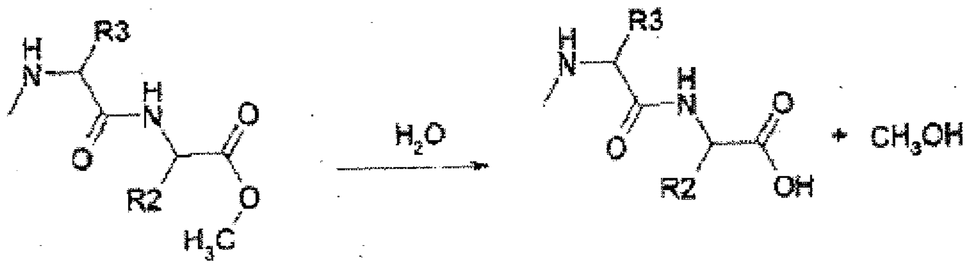
On the other hand, the esterified peptide collected from the reaction solution was subjected to the final reaction.

(iii) The final reaction sub-step for conversion of the esterified peptide (peptidyl methyi ester) into the peptide with a free carboxyl group at its C-terminus:

10 % DMAE aqueous solution was used at 60 °C for 20 min in the hydrolysis reaction of the ester bond to convert the esterified peptide (peptidyl methyi ester) into the peptide with a free carboxyl group.

The reaction of hydrolysis of ester may be carried out by the following reaction scheme:

Hydrolysis of ester:



The peptide with a free carboxyl group was collected from the aqueous solution, and then was dried up to use as a dried peptide sample for the next degradation step.

Accordingly, the peptidyl reaction product (peptide with a free carboxyl group), which is obtained in each of the C-terminal degradation steps, is by no means analyzed by mass spectroscopy.

The C-terminal sequence of the denatured protein was made based on the HPLC analysis of the C-terminal amino acid (N-(pentafiuoropropanoyl) awlno ac~dicm ethyl ester) obtained in the sub-step (ii).

Therefore, the dried peptide sample, which has the C-terminal amino acid of -NH-CH(**R2**)-COOH to be analyzed in the next step of the C-terminal degradation, should be free from such contamination of the denatured protein that retains un-reacted C-terminal amino acid of -NH-CH(**Rl**)-COOH.

At the least, Tsugita fails to teach any process for preparation of such a mixture comprising the denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence. Tsugita by no means uses FAB-MS or MALDI-TOF-MS for the process disclosed in 2.13 C-terminal sequencing.

Further, Tsugita also teaches another process for multi-point C-terminal sequencing (i.e. Chemical specific cleavage and multiple C-terminal sequencing) for the protein, which process is carried out on the dried protein as well as on the polyacrylamide gel.

The process for the multi-point C-terminal sequencing for the protein sample on the polyacrylamide gel comprises the following steps (a) - (c):

(a) Step of electro-blotting the protein on the polyacrylamide gel to the Immobilon-CD membrane:

At first, the proteins were subjected to one-dimensional or two dimensional electrophoresis on the polyacrylamide gel. The resulted protein spots on the polyacrylamide gel were electroblotted to the Immobilon-CD membrane and negatively strained. The protein spot identified on the Immobilon-CD membrane was excised and cut into a 1 mm square.

(b) Step of chemical specific cleavage of protein on the blotted membrane:

The cut-off square piece of the blotted membrane was put in the small tube and subjected to the specified cleavage reactions.

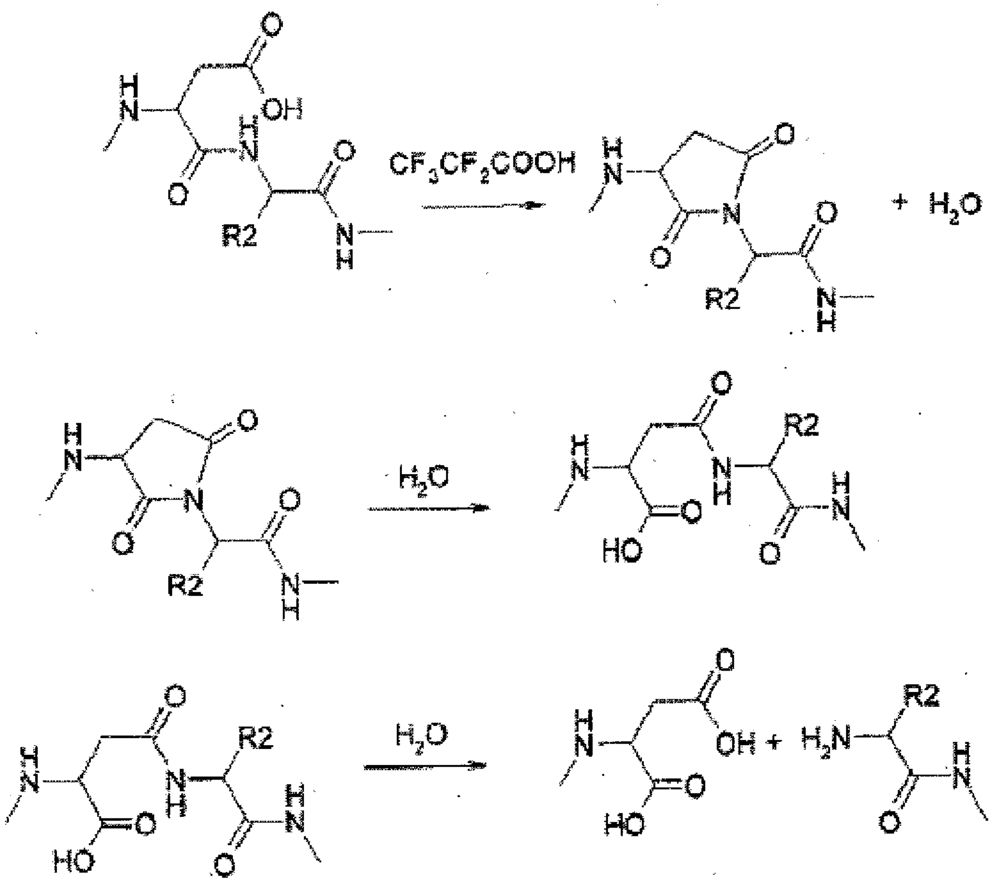
In the specified cleavage reactions, the protein sample was cleaved at the carboxyl side of the aspartyl pepdide bond (Asp-C), or at the amino side of the serine or threonine (Ser/Thr-N) peptide bonds, under the specified cleavage conditions, respectively.

Tsugita employed such a specified cleavage condition for the Asp-C cleavage reaction that a vapor phase reaction was made with a vapor generated from a 0.2 % PFPA aqueous solution containing 1 % w/v DTT at 90 °C for 4-16 h.

The group of peptidyl reaction products (peptide fragments) produced in the reaction of cleavage at the carboxyl side of the aspartyl pepdide bond (Asp-C) will consist of the N-terminal

peptide fragment having a newly exposed C-terminal aspartic acid, inner peptide fragments having a newly exposed C-terminal aspartic acid and the C-terminal peptide fragment.

The Asp-C cleavage reaction may be made through the following reaction scheme.

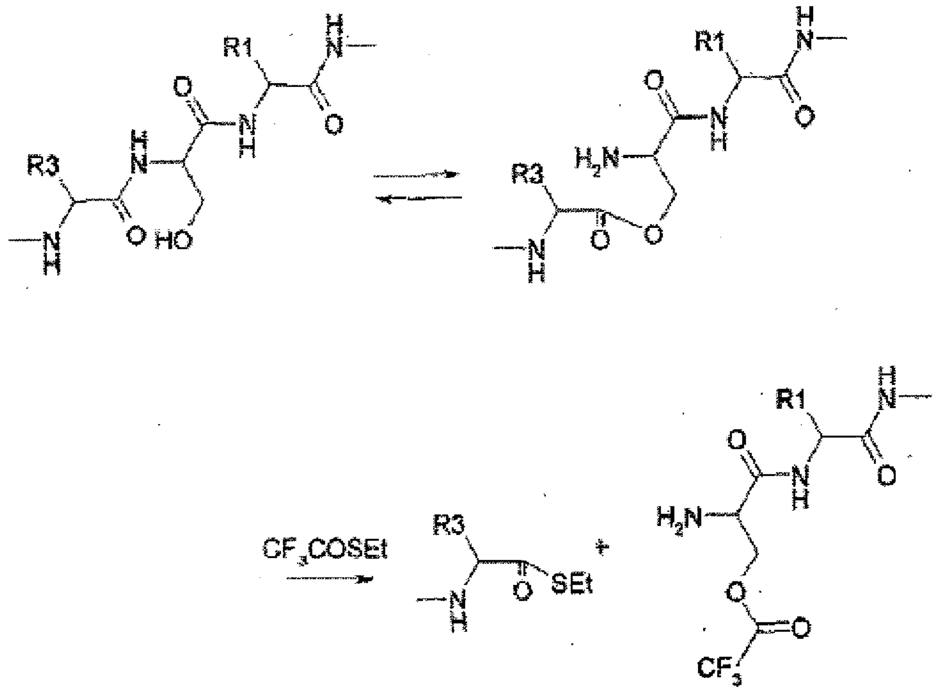


Tsugita teaches a specified cleavage condition for the See/Thr-N cleavage reaction where the vapor phase reaction was conducted with a **vapor** of TFASEt (S-Ethyl trifluorothioacetate; CF_3CO-S-CH_2CH_3) at 30 °C for 24 h or at 50 °C for 6-24 h.

The group of peptidyl reaction products (peptide fragments) produced in the reaction of cleavage at the amino side of the serine or threonine (Ser/Thr-N) peptide bonds will consist of the N-terminal peptide fragment, inner peptide fragments having a newly exposed N-terminal

Ser/Thr residue and the C-terminal peptide fragment having a newly exposed N-terminal Ser/Thr residue.

The Ser/Thr-N cleavage reaction may be made through the following reaction scheme.



(c) Step of extraction of the peptidyl reaction products from the cut-off square peace of the membrane.

After the specified cleavage reaction, the peptidyl reaction products (peptide fragments) were extracted with 30% and 60% acetonitrile aqueous solutions. The extract was dried and analyzed by FAB-MS or MALDI-TOF-MS.

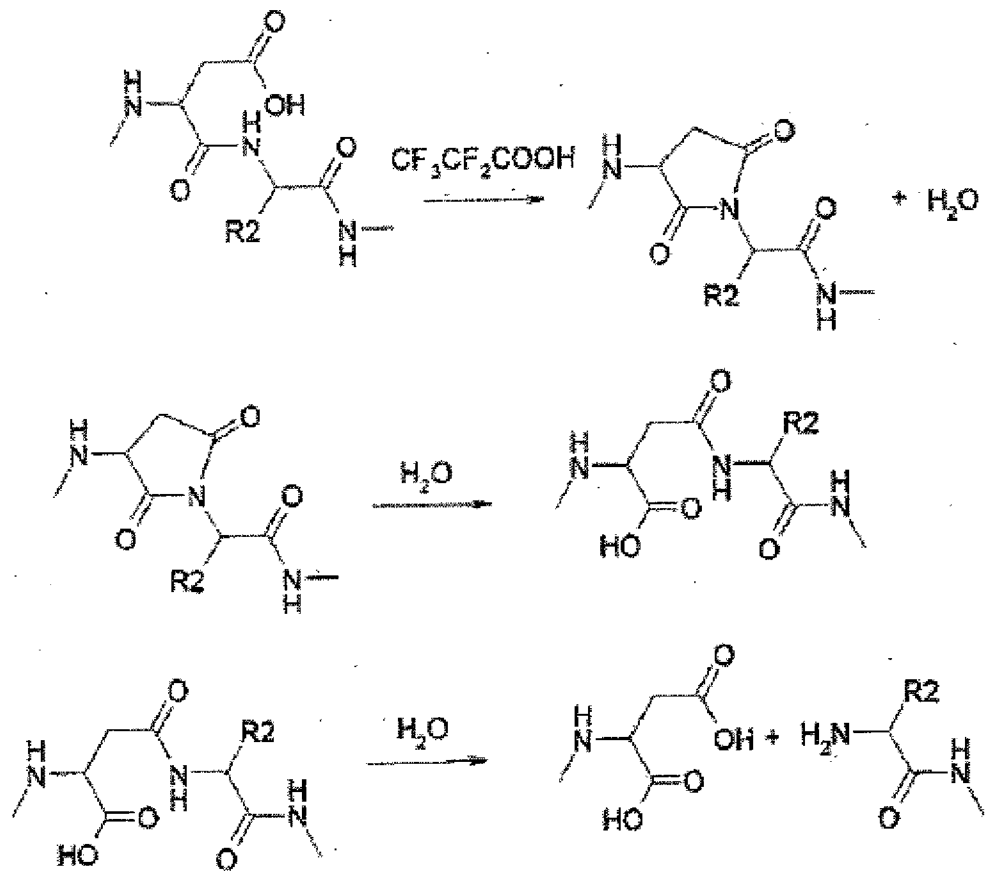
Tsugita fails to provide any suggestion as to such a process for C-terminal stepwise degradation, which is suitably used for preparation of such a mixture comprising the denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence.

Further, Tsugita fails to provide any suggestion as to whether or not PFPA, which is suitably used in the vapor phase reaction for the Asp-C cleavage reaction, would be employed as a reactant for the liquid phase reaction for degradation of the oxazolone-ring, in place of PFPMe. At least, the function of PFPMe used in the liquid phase reaction for degradation of the oxazolone-ring is quite different from the catalytic function of PFPA used in the vapor phase reaction for the Asp-C cleavage reaction. Therefore, there is no good reason to believe that PFPA would have a similar function to that of PFPMe used in the liquid phase reaction for degradation of the oxazolone-ring.

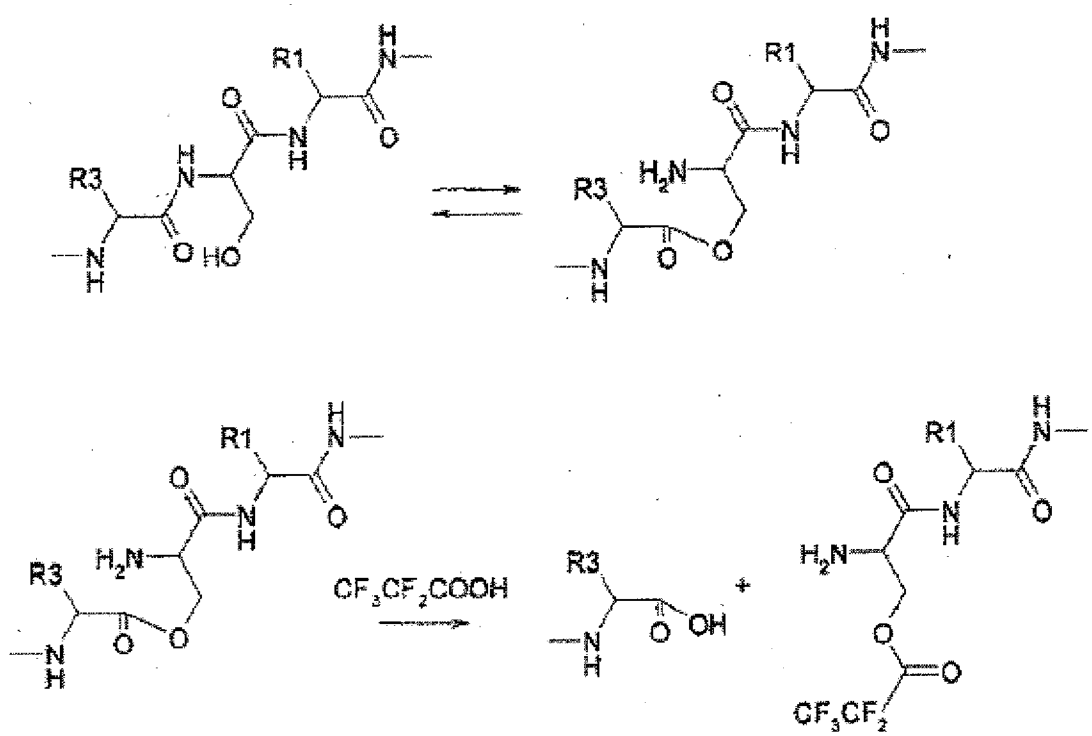
Furthermore, Tsugita also teaches another process for C-terminal sequencing at multiple sites for the protein, where the process is carried out on the dried protein sample as well as the protein sample blotted on Immobilon-CD membrane. (See 3.4 C-terminal sequencing at multiple sites).

In the process for C-terminal sequencing at multiple sites, the dried protein sample or the protein sample blotted on Immobilon-CD membrane was reacted with the vapor of concentrated perfluoric acid, i.e., a vapor from a 90 % PFPA aqueous solution containing 1 % DTT at 90 °C for 2-16 h. The reaction with the aqueous vapor from 90 % PFPA aqueous solution at 90 °C for 2-16 h provided cleavage at the C-side of aspartic acid (Asp-C cleavage reaction) and cleavage the N-side of serine/threonine (Ser/Thr-N cleavage reaction), and simultaneous successive truncation at the C-terminus of the cleaved fragments.

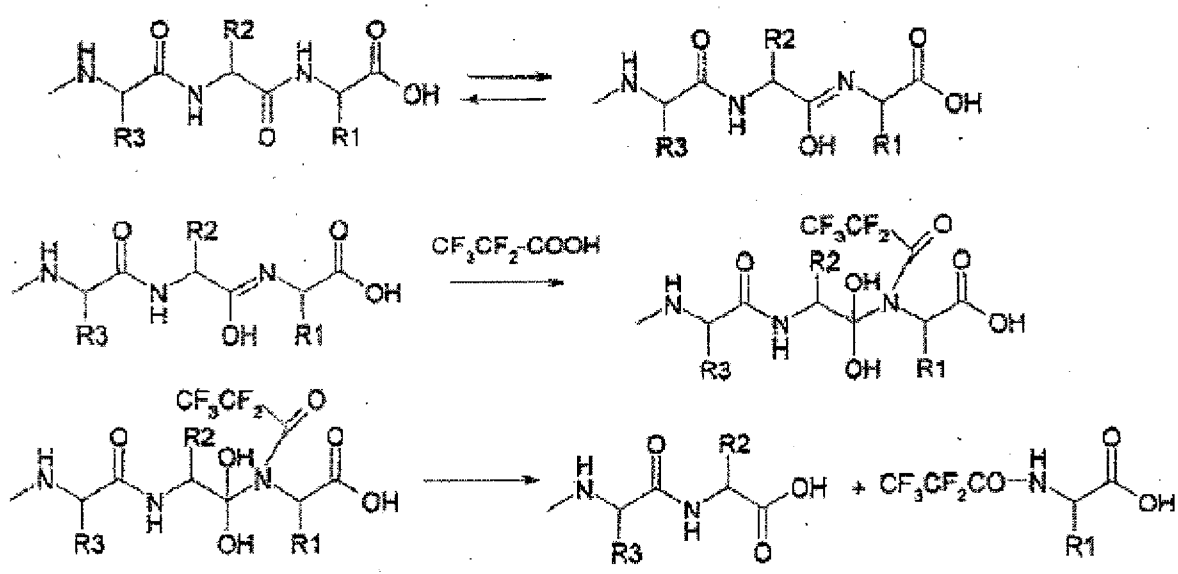
The Asp-C cleavage reaction may be made through the following reaction scheme.



The Ser/Thr-N cleavage reaction may be made through the following reaction scheme.



The reaction of the simultaneous successive truncation at the C-termini of the cleaved fragments may be made through the following reaction scheme.



In the reaction of truncation at the C-terminus of the cleaved fragment, the vapor of PFPA (CF_3CF_2COOH) may be used as a reagent.

As shown in Table 3, the C-terminal Pro of the fragment of SPRESLSALP was successfully truncated by using the reaction condition, and thus, the formation of oxazolone-ring is by no means contained in the reaction path. Indeed, as Pro has a cyclic form, any oxazolone-ring can never be formed from Pro residue at the C-terminus of the peptidyl fragment.

Therefore, the reaction scheme of the Tsugita process used for C-terminal sequencing at multiple sites is concluded to be quite different from the reaction scheme of the process as described in Claim 1.

Accordingly, Tsugita fails to teach any process for preparing a mixture containing a series of peptidyl reaction products by chemically releasing the C-terminal amino acids successively, in which the oxazolone-ring is formed from the C-terminal amino acid, and any chemical cleavage of the peptide is successfully prevented in the chemical reaction step.

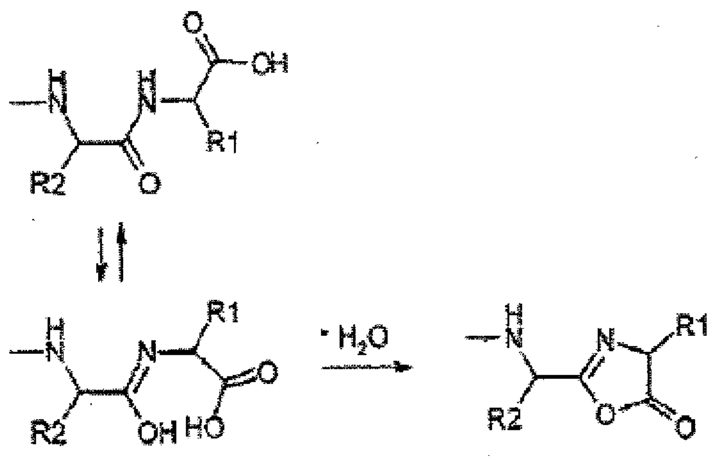
In addition, Tsugita fails to teach any process in which chemical specific cleavage was carried out on the protein being maintained in a state that it is bound on the polyacrlamide gel in place of the protein on the blotted membrane.

Tsugita fails to provide any suggestion as to such a process for C-terminal stepwise degradation or for chemical specific cleavage, which is carried out on the peptide (denatured protein) being maintained in a state that it <u>is</u> bound on the gel carrier such as polyacrlamide gel.

In contrast, the process for releasing the C-terminal amino acids successively from the peptide of the present invention is carried out through the following reaction schemes:

(I) reaction for formation of 5-oxazolone ring:

The reaction for formation of 5-oxazolone ring is expressed on the whole by the following reaction scheme (I):

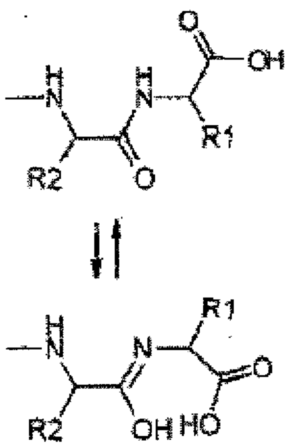


(I)

The reaction of scheme (I) consists of the following two stages (Ia) and (Ib).

(Ia) keto-enol tautomerism:

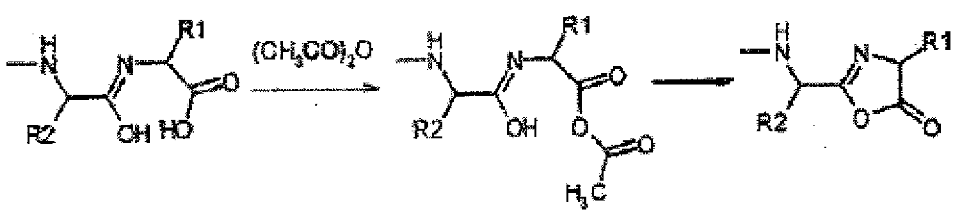
The perfluoroalkanoic acid contained in the mixed solution of the alkanoic acid anhydride and the perfluoroalkanoic acid dissolved in the dipolar aprotic solvent is allowed to act as a proton donor on the dried peptide at the stage of keto-enol tautomerism, as shown in the following reation scheme (Ia):



(Ia)

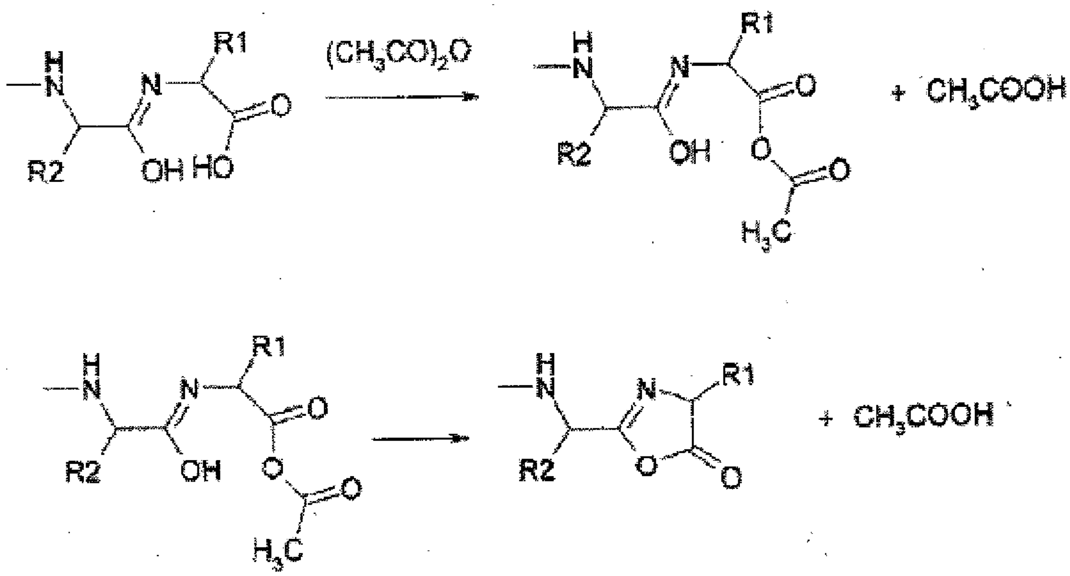
(Ib) formation of the activated C-terminal carboxyl group and formation of the intra-molecular ester bond (formation of the 5-oxazolone ring):

The alkanoic acid anhydride is used as a reagent for formation of the activated C-terminal carboxyl group. The activated C-terminal carboxyl group is reacted with the hydroxyl group to form the 5-oxazolone ring.



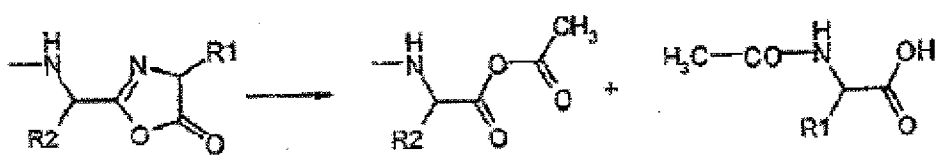
(Ib)

The following is a detailed reaction scheme of the stage (Ib):



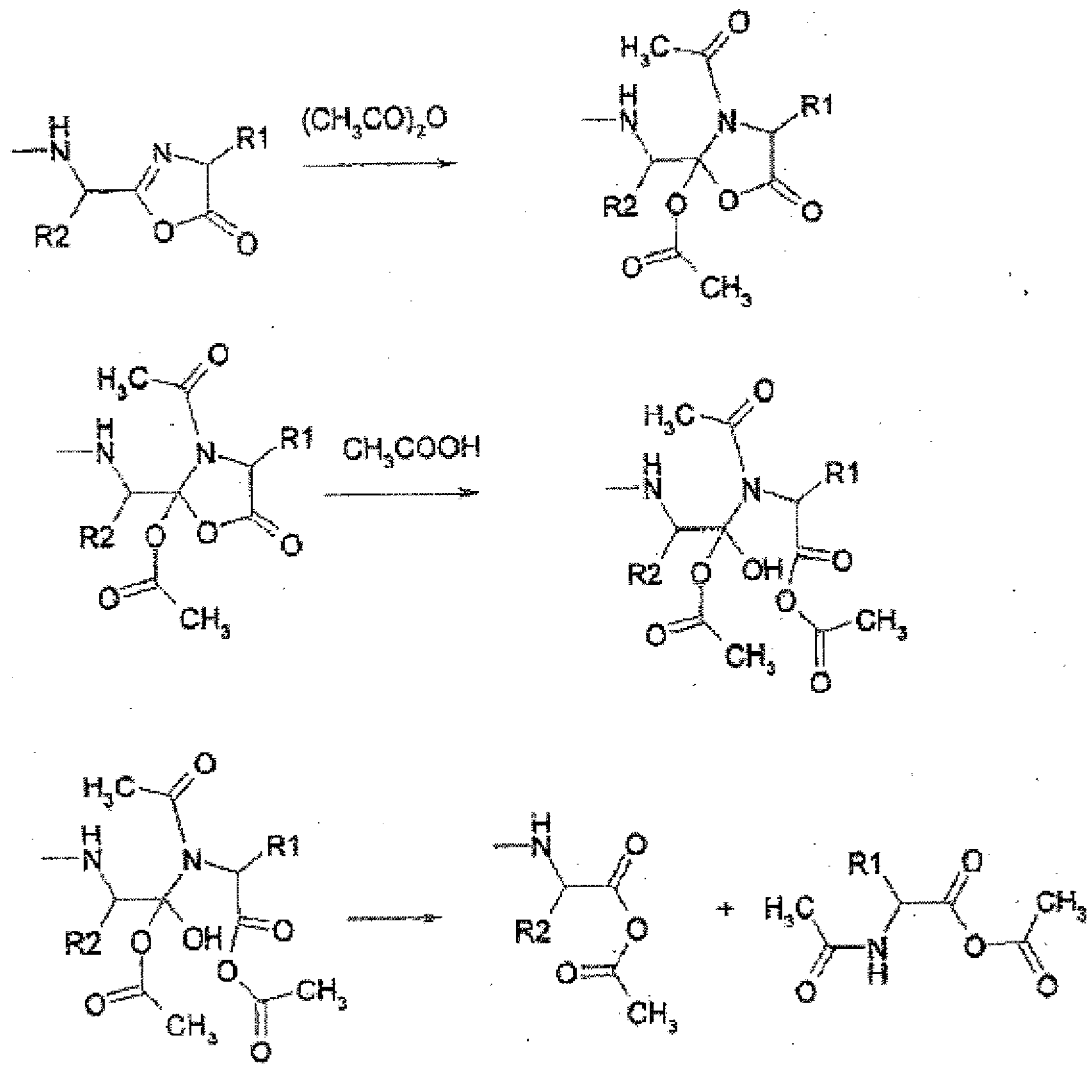
(II') separation of the C-terminal amino acid and formation of the reaction intermediate for the next stage:

The alkanoic acid anhydride is used as a reagent for the addition reaction on the double bond of >C=N- type of the 5-oxazolone ring. The degradation of the 5-oxazolone ring is made via such a reaction as shown by the following reaction scheme (II'):



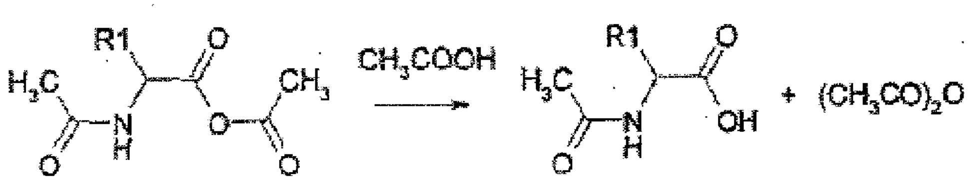
(II')

The following may be a detailed reaction scheme of the stage (II')



The alkanoic acid, which is a by-product from the alkanoic acid anhydride formed at the stage (Ia) is used as a reagent at the second reaction for opening of the ester bond therein.

In addition, the alkanoic acid also reacts on the derivative of the C-terminal amino acid having acid anhydride form, and thereby the C-terminal acid anhydride form thereof is converted into the C-terminal carboxyl group.



The peptidyl reaction product having the activated C-terminal carboxyl group of the third reaction is just ready for the formation of the 5-oxazolone ring at the next stage.

The considerable variation of the reaction speeds of those stages is successfully used to prepare a mixture comprising the original peptide and the series of peptidyl reaction products produced therefrom.

At least, the reaction schemes used in the process for releasing the C-terminal amino acids successively from the peptide of the present invention are quite different from those used in the process for C-terminal stepwise degradation or in the process for C-terminal sequencing at multiple sites disclosed in Tsugita.

Covey teaches a procedure of enzymatic digestion of the long peptide by trypsin to cleave the long peptide into tryptic fragments. Covey also teaches such a double charge rule that the tryptic fragment having Arg or Lys at the C-terminus thereof will be doubly positively charged by using Ion spray process for Ion Evaporation Mass Spectrometry, but that there are three exceptions to the double charge rule as follows:

First exception: such a tryptic fragment having other amino acid than Arg or Lys at the C-terminus thereof will only be singly charged by using Ion spray process for Ion Evaporation Mass Spectrometry.

Second exception: such a tryptic fragment having an amino terminus which is carboxylated or blocked (e.g. N-acylation at the N-terminus) will only be singly charged by using Ion spray process for Ion Evaporation Mass Spectrometry.

Third exception: such a tryptic fragment having Arg or Lys at the C-terminus thereof and containing an <u>internal HIS</u> will be triply charged in small percentage, but will be doubly charged in most percentage.

However, Covey fails to provide any suggestion as to whether or not such a double charge rule will be also observed for MALDI-TOF-MS or FRB-MS.

At the least, Covey fails to provide any suggestion as to intensity of a singly positive charged ion from the tryptic fragment having Arg or Lys at the C-terminus thereof to be measured by MALDI-TOF-MS. Further, Covey fails to provide any suggestion as to intensity of a singly negative charged ion from the tryptic fragment having other amino acid than Arg or Lys at the C-terminus thereof to be measured by MALDI-TOF-MS.

At the least, Covey fails to provide a teaching that a singly positive charged ion from the tryptic fragment having Arg or Lys at the C-terminus thereof may show stronger intensity in cationic species spectrum measured by MALDI-TOF-MS.

Xu teaches a procedure of structural characterization of muropeptides derived from peptidoglycan by means of MALD-MS based method. Xu teaches the MALDI-TOF-MS spectra measured for the muropeptides derived from peptidoglycan by using lysostaphin digestion such as the positive-ion linear MALDI mass spectra shown in Figure 2, in which $[M+Na]^+$ ion peaks are the dominant species, but $[M+H]^+$ ion peaks are not measured at any detectable level.

In particular, in MALDI-PSI3 analysis of an un-substituted monomer, Xu teaches in positive-ion mode analysis, the $[M+H]^+$ ion at m/z 991 was selected as the precursor for an unsubstituted muropeptide monomer because of its high abundance relative to the protonated molecules $[M+H]^+$.

In view of these teachings, Xu fails to suggest any use of the protonated molecules $[M+H]^+$ for the positive-ion mode analysis. Xu fails to provide any suggestion that the protonated molecules $[M+H]^+$ will be used for structural characterization of peptides in combination with the deprotonated molecules $[M-H]^-$.

The combination of Tsugita, Covey and Xu fail to teach at least the following elements of Claim 1, from which Claims 2 and 4-6 depend. The combination of references fail to teach, at the least, any process for preparation of a mixture comprising the denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence. The combination of references do not teach FAB-MS or MALDI-TOF-MS for the process disclosed in 2.13 C-terminal sequencing. Further, the combination of references fail to teach a process for C-terminal stepwise degradation, which is suitably used for preparation of such a mixture comprising the denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence.

Thus, it is respectfully requested that the rejection of Claims 1, 2 and 4-6 under 35 U.S.C. §103(a) be withdrawn.

Claim 3 stands rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Tsugita in view of Covey and Xu, in further view of Harris. The deficiencies of Tsugita, Covey and Xu are discussed above. Harris does not cure these deficiencies. This rejection should be withdrawn based on the comments and remarks herein.

Harris teaches a method of using the $[M+H]^+$ ions from the trypsin autolysis fragments as mass calibrants in the positive-ion mode MALDI-TOF based analysis.

However, Harris fails to teach that the $[M-H]^-$ ions from the trypsin autolysis fragments will be successfully used as mass calibrants in the negative-ion mode MALDI-TOF based analysis.

At the least, Harris fails to provide any teaching that the $[M-H]^-$ ions from the trypsin autolysis fragments will be successfully measured in the negative-ion mode MALDI-TOF based

analysis. None of the other references, including Tsugita, Covey and Xu teach this element, which is recited in Claim 3.

Further, the combination of Tsugita, Covey, Xu and Harris fail to teach at least the following elements of Claim 1, from which Claim 3 depends. The combination of references fail to teach, at the least, any process for preparation of a mixture comprising the denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence. The combination of references do not teach FAB-MS or MALDI-TOF-MS for the process disclosed in 2.13 C-terminal sequencing. Further, the combination of references fail to teach a process for C-terminal stepwise degradation, which is suitably used for preparation of such a mixture comprising the denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence.

Thus, it is respectfully requested that the rejection of Claim 3 under 35 U.S.C. §103(a) be withdrawn.

Claims 7-17 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Tsugita in view of Covey and Xu and in further view of Vogt. The deficiencies of Tsugita, Covey and Xu are discussed above and herein below. Vogt does not cure these deficiencies. This rejection should be withdrawn based on the comments and remarks herein.

Tsugita fails to teach any process in which chemical specific cleavage was carried out on the protein being maintained in a state that it is bound on the polyacrlamide gel in place of the protein on the blotted membrane.

Accordingly, Tsugita fails to provide any suggestion as to such a process for C-terminal stepwise degradation or for chemical specific cleavage, which is carried out on the peptide

(denatured protein) being maintained in a state that it is bound on the gel carrier such as polyacrlamide gel.

The process as recited in Claims 7-17 employs a liquid phase reaction in place of a vapor phase reaction. At the least, the reaction schemes used in the process for releasing the C-terminal amino acids successively from the peptide are quite different from those used in the process taught by Tsugita.

Vogt teaches a process for preparation of a high reactive gel-suspension of carboxymethyl cellulose (CIVIC), in which the polymer (carboxymethyl cellulose) is treated in a dipolaraprotic solvent, such as N, N-dimethylacetamide and dimethylsulfoxide (DMSO), with p-toluene-sulfonic acid.

Vogt also teaches a mechanism where the activation (swelling in the dipolar-aprotic solvent) is achieved via an interaction between the carboxylate groups ($-CH_2-COONa$) of Na-CMC and $H0_3S$-groups of the p-toluene-sulfonic acid with a rapid exchange of the acidic hydrogen as well as an interaction of the lipophilic toluene unit of the p-toluene-sulfonic acid with the solvent.

Accordingly, Vogt fails to teach any process for preparation of gel-suspension of CMC in the dipolar-aprotic solvent without p-toluene-sulfonic acid. Vogt also teaches that an effective method for activation of CMC is precipitation of an aqueous solution of CMC by N,N-dimethylformamide (DMF) and the removal of the water from the swollen gel by repeated distribution under reduced pressure. This statement teaches that N,N-dimethylformamide (DMF) can never remove water from the water-swollen gel of CMC.

Further, Vogt teaches that other acids like methane sulfonic acid, triflioroacetic acid and monochloroactic acid do not swell CMC to a comparable extent. Furthermore, Vogt teaches that

polysaccharides at the polymer backbone bound carboxy groups like sodium alginate, sodium pectinate, and 6-carboxy cellulose do also not swell in the manner described for CMC.

Vogt fails to provide any teaching as to whether or not the combinational use of the dipolaraprotic solvent with other acid than p-toluene-sulfonic acid is successful for preparation of non-aqueous swelling gel of polymer other than CMC.

Further, Vogt fails to provide any suggestion as to whether or not the use of the dipolar-aprotic solvent without p-toluene-sulfonic acid is successfully applied for preparation of non-aqueous swelling gel of other polymer than CMC.

At the least, Vogt fails to provide any suggestion as to whether or not the combinational use of the dipolar-aprotic solvent with perfluoroalkanoic acid is successfully applied for preparation of non-aqueous swelling gel of polymers other than CMC. None of the other references, including Tsugita, Covey and Xu teach this element, which is recited in Claims 7-17.

Further, the combination of Tsugita, Covey, Xu and Vogt fail to teach the following elements of Claim 1, from which Claims 7-17 depend. The combination of references fail to teach, at the least, any process for preparation of a mixture comprising the denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence. The combination of references do not teach FAB-MS or MALDI-TOF-MS for the process disclosed in 2.13 C-terminal sequencing. Further, the combination of references fail to teach a process for C-terminal stepwise degradation, which is suitably used for preparation of such a mixture comprising the denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence.

Further, the combination of references fail to teach at least the following element of Claim 7. The combination of references fail to teach any process for C-terminal sequencing, in which the reactions for C-terminal stepwise degradation are carried out for the peptide being maintained in a state that it is bound on the gel carrier.

Thus, it is respectfully requested that the rejection of Claims 7-17 under 35 U.S.C. §103(a) be withdrawn.

For at least the reasons set forth in the foregoing discussion, Applicants believe that the Application is now allowable, and respectfully requests that the Examiner reconsider the rejection and allow the Application. Should the Examiner have any questions regarding this Amendment, or regarding the Application generally, the Examiner is invited to telephone the undersigned attorney.

Respectfully submitted,

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